

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14593 A2

- (51) International Patent Classification⁷: C12Q 1/70 (81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, VZ, VN, YU, ZA, ZW.
- (21) International Application Number: PCT/EP00/07892
- (22) International Filing Date: 14 August 2000 (14.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
A 1443/99 20 August 1999 (20.08.1999) AT
- (71) Applicant: BAXTER AKTIENGESELLSCHAFT
[AT/AT]; Industriestrasse 67, A-1220 Vienna (AT).
- (72) Inventors: ZERLAUTH, Gerold; Sobieskigasse 42/9, A-1090 Vienna (AT). GESSNER, Mathias; Johann Nestroygasse 12/19, A-2301 Gross Enzersdorf (AT). KOETTINITZ, Karl; Bockfliesserstrasse 184/4, A-2232 Deutsch Wagram (AT). GROSS, Patricia; Heiligenstaetterstrasse 33/23, A-1190 Vienna (AT).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR PRODUCING QUALITY ASSURED BIOLOGICAL SAMPLE AND COMPOSITION CONTAINING THE SAME

(57) Abstract: A method for producing a pool of biological samples that is quality assured with respect to the load with microorganisms, using a nucleic acid amplification method, in which a screening pool is tested with a high sensitivity nucleic acid amplification method and can be divided into screening subpools, which are tested with a less sensitive nucleic acid amplification method, where individual samples can be picked out and eliminated, is described.

WO 01/14593 A2

A METHOD FOR PRODUCING QUALITY ASSURED BIOLOGICAL SAMPLE AND COMPOSITION CONTAINING THE SAME

The invention relates to a method for producing a pool of biological
5 sample with assured quality with respect to the load of microorganisms, using
nucleic acid amplification methods.

Biological samples, such as plasma donations or batches of cell culture
supernatants, can be contaminated with undesired microorganisms, especially
viruses or foreign DNA. These contaminations can lead to undesired reactions
10 in preparations that are produced from such biological preparations and are
administered to humans.

Above all, human plasma is of extraordinary clinical importance as a
starting material for producing plasma derivatives, especially for substitution
therapy in a hereditary or acquired deficiency of plasma components. Pharma-
15 ceutical preparations of this kind as a rule are not produced from single
donations, but rather from a plasma pool consisting of a very large number of
individual donations.

However, when using human plasma care must be taken that it does not
contain any infectious agents that could be transferred with the pharmaceutical
20 preparation or with the plasma derivatives. Among infectious agents that could
possibly be in the blood are above all viruses that can be transferred through the
blood (hematogenic viruses), for example, HI-viruses or hepatitis viruses (A, B,
C, D, E or G) as well as parvovirus.

Because of the great demand for drugs that contain plasma derivatives
25 economical production of these drugs is possible only on an industrial scale.

Plasma is obtained from donors and pooled for the production of
pharmaceutical preparations. A usual pool consists of about 2000-6000
individual plasma donations. Here there is the risk that the total plasma pool
will become contaminated by a single virus-contaminated plasma donation.
30 Although there was already success in processing human albumin to a virus-
safe preparation by heating by the end of the first half of the 20th century, this

was initially not possible with all other drugs obtainable from plasma because of their sensitivity to heat. To this day, although there have been millions of uses of adequately prepared albumin preparations, there have never been infections with viruses occurring in the blood in humans.

5 In contrast, several viral infections, especially with hepatitis viruses, have been reported with many other drugs produced from plasma and since the 1980s there have been increasing reports of infections with the AIDS virus.

Around 1980 heat treatments were carried out for the first time with the appropriately stabilized factor VIII concentrates with the intention of achieving
10 inactivation of viral contaminants by this. However, at first one had to accept a large loss of factor VIII activity and the actual inactivation potential remained unknown, respectively.

Finally, through improvement of the heat inactivation methods and the use of other new inactivation methods it was possible to produce drugs from
15 plasma that in most cases did not lead to viral infections in the receiver. This development also went hand in hand with improvement of the donor and donation selection with the goal of excluding every donor and donation in which the possibility of viremia and thus a virus-containing plasma existed.

Since a long time it has been attempted either through detection of
20 antigens or antibodies to or against a certain virus in the blood to exclude those donations which give a positive result and not to introduce them into a larger plasma pool that is intended to serve as starting material for the production of blood products. In the case of individual donors who in all tests do not have any symptoms of disease or pathological test results even though certain viruses can
25 be present in their blood even in high concentration for a prolonged period of time. Now the occurrence of such viremias by a specific virus can be unambiguously detected with the aid of an amplification method.

A single plasma donation contaminated with viruses does of course become diluted through the pooling of plasma units, but the detection of viral
30 genome sequences with the aid of amplification reactions is so sensitive that

even in these dilutions virus genomes or their sequences respectively are unambiguously determinable, and if they, as mentioned above, fall under a certain detection limit, then they no longer have any clinical relevance as far as the possibility of an infection is concerned.

5 The EC guideline in accordance with the "EEC Regulatory Document Note for Guidance," Guidelines for Medicinal Products Derived from Human Blood and Plasma" (Biologicals 1992, 20: 159-164) proposes a quality assurance system for the control of plasma donors or plasma donations. Accordingly, every plasma donation has to be tested with validated tests for the
10 absence of viral markers such as hepatitis B antigen, HIV-1 and HIV-2 antibodies, since these are an indication of a corresponding viral infection of the plasma donor. Tests to exclude hepatitis C infection are also to be conducted.

 According to the European Pharmacopoeia, special tests are supposed to be carried out to determine hepatitis B surface antigen, for hepatitis C virus
15 antibody and for HIV antibodies in every donation (European Pharmacopoeia, 2nd Edition, Part II, 1994, pp. 853-4).

 The FDA guideline of March 14, 1995 provides a PCR (Polymerase-Chain-Reaction) testing of an end product (immunoglobulin product) as an additional safety factor.

20 In spite of the proposed tests, it is stressed in the EC guideline that the safety of individual plasma donations by a control of all these virus markers alone is not sufficient. Even if the absence of the said markers in a plasma sample is confirmed, viremia of the donor cannot be excluded. Viral antigens and the corresponding antibodies are not always immediately detectable after an
25 infection. The first markers for a viral infection mostly do not arise until weeks or months after a contact with an infectious material. This critical period of time after an infection and before the occurrence of antibodies is generally characterized as a "window period." The time after infection in which the first viral markers are detectable, however, varies from virus to virus.

Moreover, it is also known that for many drugs produced from plasma a depletion or inactivation occurs as part of the production process and such products themselves are virus safe to a great extent.

Although virus inactivation of plasma derivatives have been carried out
5 extremely successfully on an industrial scale. Nevertheless in rare cases there have been transmissions of hematogenic viruses like AIDS, hepatitis A, B and C. Therefore it has to be assumed that virus-contaminated products have been produced by the manufactures in a few production batches in spite of a constant production method (Lancet 340, 305-306 (1992); MMWR 43 (28), 505-509
10 (1994); Thromb. Haemost. 68, 781 (1992)). The cause of this should probably be sought in an extremely high contamination of certain starting batches. Since only indirect methods are available to exclude virus-contaminated plasma donations in producing the plasma, there is the possibility that the starting material will be so highly contaminated that the otherwise successful virus
15 inactivation and virus depletion methods are no longer sufficient to produce virus-safe end products.

Since the human infectious dosage for most human pathogenic viruses is unknown and at the present is not even determinable in testing the plasma pool, it is desired to pick out every single contamination by identifying the
20 contaminated individual samples. The nucleic acid amplification tests that are used therefore are indeed extremely sensitive, but very costly. So their use is justified with known human pathogenic viruses, while for other microorganisms of which either no human pathogenic effect is known or described or for which only a very high pathogen dosage is infectious, this expenses would not be
25 necessary.

In particular, WO 96/35437 and US 5 591 573-A describe test systems for plasma pools using nucleic acid amplification methods and/or antibody testing. US 5 591 573 discloses a method for testing plasma pools. With this method a first pool is prepared and tested by means of PCR. If this PCR test is
30 positive, a second smaller subpool is prepared and again tested by means of

PCR. This process is repeated until the contaminated individual donation is identified. Antibody testing or maintenance of preset definite limit values, disclosed as part of the screening method, are also envisioned in accordance with WO 96/35437.

5 However, the described nucleic acid amplification methods have in common the fact that one always attempts to use the amplification method that allows the greatest sensitivity. However, as noted, these high sensitivity PCR tests are extremely costly, in particular in serial testing of pools of individual biological samples. PCR tests with lower sensitivity, which would be far more
10 cost favorable, however, are generally avoided in such testing, since the risk of contaminations that lie under the (low) detection limit would be concomitantly high.

 Similarly, even with supernatants from recombinant cell cultures contaminations by microorganisms or by nucleic acid material from host cells
15 continue to occur. Such contaminations should, of course, not be present or should be present only at a certain maximum value (limit value) in pharmaceutical preparations that are to be purified from the supernatants.

 The task of this invention is thus to make available a method for producing a pool of biological samples using nucleic acid amplification
20 methods that is quality assured with regard to the load of microorganisms, especially viruses, a method which on the one hand enables a reliable identification of contaminated individual samples, especially highly contaminated individual samples, as well as adherence to certain limit values for such contaminants in the pool, but on the other hand brings a cost reduction and
25 a method that is simpler to the pool testing methods known from the prior art.

 In accordance with the invention, this task is solved by a method for producing a pool of biological samples that is quality assured with regard to the load of microorganisms using a nucleic acid amplification method which is characterized by the following steps:

- Taking aliquots from the biological samples,
- Combining the aliquots into a screening pool,
- Testing the screening pool for the presence or the content of genome equivalents of microorganisms by means of a first nucleic acid amplification process, which has a certain selected detection limit DL-1 with respect to the microorganisms to be tested,
- Dividing the screening pool into screening subpools by recombining the aliquots that were taken into smaller pools when a preset limit value of genome equivalents is exceeded in testing the screening pool,
- Retesting the screening subpool for the presence of or the content of genome equivalents of the microorganism that is to be tested by means of an additional nucleic acid amplification process, which has a certain selected detection limit DL-2 for the microorganisms to be tested, where $DL-1 < DL-2$,
- Identifying and eliminating those samples that exceed DL-2, and
- Combining the non-eliminated samples into a quality assured pool.

By providing for at least two nucleic acid amplification processes that differ with regard to their sensitivity in accordance with the invention an efficient (because of the high sensitivity of the pool testing) and nevertheless cost favorable method can be made available. Since testing the subpool is carried out up to the individual samples by a less sensitive nucleic acid amplification method without prior further extraction of the nucleic acid (which therefore is decisively more cost favorable and less expensive).

With the method in accordance with the invention thus in a first step the screening pool is tested for nucleic acid contamination with a very sensitive and quantitative method. The very costly determination of the contaminated individual sample or donation, which involves a very much higher number of additional tests, is determined with a less sensitive nucleic acid amplification method. In doing so, no risk is taken that a relevant contamination will be overlooked, since the screening pool testing takes place with a method that is as sensitive as possible. But on the other hand the costs and expenditures of time

of the elimination method for the contaminated individual samples or batches are decisively reduced. In those instances in which determination of such contaminated individual donations has up to now not been carried out for these reasons, the method in accordance with the invention offers for the first time the possibility to use the valuable raw material, which the individual donations represent, at low cost and keeping these samples from being discarded.

The sensitivity of the nucleic acid amplification method is set in accordance with the invention as the quantity of genome equivalents that is just still detectable with the relevant nucleic acid amplification method. Providing methods of this kind with precisely established limit values is easily possible for the man skilled in the art and is a part of his ordinary knowledge.

The method in accordance with the invention is suitable above all for quality assurance of pools with respect to the load of microorganisms, which are of low pathogenicity or toxicity or of which it can be assumed that a load that lies under the established limit value is in each case eliminated in the course of the subsequent purification process up to the preparation of the pharmaceutical preparation or that the amount of contamination corresponding to this limit value in a pharmaceutical preparation of this kind is harmless and without side effects. A particular example of this consists of contaminations with parvoviruses, in particular parvo B19.

Parvovirus B19 is a single strand DNA virus with 32 nm diameter that does not have a lipid membrane and thus is relatively resistant to virus inactivation methods. Thus, most inactivation methods, for example, physical methods like pasteurization (60°C over 12 h) or chemical treatments like organic solvents (TNBP and/or detergents) do not have satisfactory results. Only dry heat treatment appears to have proven to be effective.

The parvovirus B19 is the causative agent of the harmless infectious erythema (erythema infectiosum), with arthralgias and arthritis occasionally being observed. Intrauterine infections are feared, since they often end with fetal death. The limited circle of B19 threatened patients include those with

chronic hemolytic anemia, patients following bone marrow transplantations, patients with congenital/acquired immune deficiency as well as pregnant women (Sibrowski et al., Beitr Infusionsther. Basel, Karger, 1990, 26, 22-26).

5 The seroprevalence in industrial countries is 2-10% in children under 5 years of age and 40-60% in adults over 20 years of age and 85% in adults over 70 years of age. This high seroprevalence thus results in a large number of positive results in plasma pool testing, thus resulting in the need for a large number of additional tests in order to determine the highly contaminated individual samples.

10 EP-A-0 922 771 describes a method for detecting high virus concentrations in blood plasma, in which the sensitivity of PCR is decreased through the use of suboptimum conditions in the extraction, amplification or detection, so that the parvovirus DNA can only still just be detected in samples whose DNA content is greater than 10^6 - 10^7 genome equivalents/mL. However, this
15 method has the disadvantage that it is not suitable for testing pools.

Nevertheless, all of the microorganisms that occur such as bacteria or viruses can be analyzed in accordance with the invention, where above all quality assurance with respect to viruses is of particular priority in the testing of blood and plasma donations.

20 Preferably, hepatitis viruses, in particular HAV, HBV, HCV, HDV, HEV and HGV, retroviruses, in particular HIV-1 and HIV-2, and parvoviruses, in particular parvo B19, are tested with the method in accordance with the invention or plasma pools are quality assured with respect to these kind of viruses.

25 In testing various batches of a recombinant production of proteins the maintenance of certain (prescribed) limit values of the contaminations with host cell nucleic acids (here eukaryotic or prokaryotic cells or DNA or RNA are detected here as microorganisms) or contamination with certain bacterial or viral contaminations is tested.

A particularly preferred variation of the method in accordance with the invention consists of setting the limit value in testing the screening pool between the detection limit of the first nucleic acid amplification method DL-1 and 10^5 genome equivalents/mL, where in particular a value around 10^4 genome equivalents/mL has proved to be particularly efficient for parvo B19, for instance. Preferably the relevant limit can also be in agreement with the relevant detection limit.

The detection limit for the first nucleic acid amplification method DL-1 preferably lies in the range of 10^2 - 10^4 genome equivalents/mL (however, it can be up to 10-50 GE/mL or, in exceptional cases, even lower), whereas the detection limit DL-2 is usually chosen to be 10^4 - 10^7 genome equivalents/mL. DL-1 and DL-2 preferably differ by at least one power of ten, but a difference of about two powers of ten has proved to be particularly efficient in accordance with the invention, since in this way the contaminated individual donations can still be reliable identified (this is necessary in accordance with the invention) and on the other hand the costs and expenses for the screening process can be reduced considerably compared to the known methods.

The amplification of nucleic acids can in accordance with the invention take place by a series of amplification processes that are described in the literature; the PCR method has proved to be particularly effective here and because of its widespread usage and industrial availability as well as its costs it is also preferred. The PCR amplification method was first described in 1983 by Mullis et al. (US 4,683,195 and US 4,683,202). Viral genome sequences can also be amplified by "nested PCR" (Methods Enzymol. 155 (1987), 335-350). For amplification and detection of RNA the RNA must first be transcribed to DNA. This method is conducted using reverse transcriptase and is called RT-PCR (Cell 50 (1987), 831-840).

The analysis of the amplification products can take place through the use of labeled nucleotides or oligonucleotide primers in an elongation process and subsequent hybridization or gel electrophoretic separation of the products.

Detection of amplified DNA fragments can take place, for example, by means of a probe that carries two fluorescence dyes. This method is described, for example, by Livak et al. (PCR Method and Appl. 1995; 4:357-362). The particular property of this probe lies in the fact that the fluorescence of the dye (FAM) attached at the 5' end of the probe, the reporter, is reduced by the presence of the second fluorescence dye (TAMRA), the quencher, which is arranged at the 3' end of the primer.

In the course of the amplification the new DNA strand is now synthesized under the effect of a thermostable DNA polymerase, preferably Taq polymerase. In doing so the DNA polymerase displaces the probe not only from the individual strand, but rather decomposes it by means of its endonucleolytic activity and thus releases the two fluorescence dyes. The fluorescence of the reporter dye is no longer suppressed by the quencher dye and increases. This increase of fluorescence is continuously recorded in the ABI prism 7700 in the course of the PCR. The threshold value, starting with which an increase of fluorescence is evaluated as a positive signal, is measured in comparison with several negative controls.

Quantification of the load with the microorganism, for example parvo B19, in an unknown sample takes place via an external calibration curve. A cloned piece of the parvo B19 DNA in a carrier plasmid, which includes the sequence to be amplified, is additionally coamplified in linearized form in concentrations of 10^6 copies/reaction up to 10 copies/reaction. By establishing a threshold value (corresponding to one PCR cycle in which the fluorescence signal of the reporter dye first rises above the baseline) a calibration line is established in the program with which the content of parvo B19 DNA of an unknown sample above its threshold value is quantified.

Alternate methods for PCR include the ligase chain reaction (LCR), in correspondence with EP 0 320 308 A and EP 0 336 731 A, and nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (SSR), in correspondence with EP 0 310 229, or the transcription based

amplification system (TAS) in correspondence with EP 0 373 960 A. A number of enzymes that can be used simultaneously or stepwise in the amplification such as a DNA polymerase or an RNA polymerase are used in these methods.

Preferably in accordance with the invention, internal standards, with
5 which the basic functioning of the amplification can be guaranteed, can be added in the nucleic acid amplification. In routine tests the interpretation of the results obtained with the method in accordance with the invention can usually be traced as follows:

a) no detection of the internal standard (i.e., no visible bands): the
10 determination did not function, for example, as a result of the amplification reaction (for example, the PCR); in this way false negative results can be excluded.

b) only the internal standard is detectable (for example, only the
standard band is visible): the determination, including the amplification reaction
15 (for example, the PCR), functioned, the sample is negative;

c) standard and sample nucleic acid are detectable (for example, both
bands are visible): positive sample.

The biological samples that are priority for testing in accordance with
the invention are selected from the group of individual blood donations, plasma,
20 serum or cell culture batches. The expected contaminations occur particularly with these biological samples and these are also used with priority in the industrial manufacture of drugs from biogenic sources.

The method in accordance with the invention can be carried out in an
automated version using robotic control, with conventional platforms being
25 particularly suitable for this. The man skilled in the art is aware of many different possibilities for producing such screening pools. For example, Mortimer (Vox Sang. 1997, 73:93-96) describes two dimensional (2D), three dimensional (3D) and even four dimensional (4D) pools. In order to resolve, for example, a 3D pool produced from 512 (8x8x8) individual samples now, one

has to use, for example, 24 subpools in order to be able to identify unambiguously a contaminated individual sample.

In accordance with the invention, the second nucleic acid amplification process is preferably carried out directly from the subpool after the first nucleic acid amplification. This means that no additional extraction of the nucleic acid
5 takes place, but rather it is determined directly in the subpool.

In accordance with another aspect, this invention also concerns a quality assured pool of biological samples, which can be obtained through the method in accordance with the invention, as well as a pharmaceutical preparation that is
10 produced or can be produced on the basis of a quality assured preparation in accordance with the invention. These pharmaceutical preparations preferably contain at least one component selected from the group of proteins, in particular plasma proteins as well as recombinantly produced proteins, and enzymes.

The invention is illustrated in more detail below by means of the
15 following example, to which it, however, is not intended to be limited.

Example

Pooling

Aliquots of 512 plasma donations are pooled by means of a three
20 dimensional scheme to a screening pool and 24 subpools.

Extraction

The nucleic acid extraction from a screening pool is carried out by means of a modified protocol of the QIAmp viral kit from QIAGEN. For this 1
25 mL of the screening pool is subjected to ultracentrifugation, the supernatant is reduced to 140 μ L. After the addition of 560 μ L AVL lysis buffer in accordance with the QIAGEN kit the mixture is incubated for 10 min at 56°C in a thermal shaker. The lysate is applied to a silica column and forced through the column by centrifuging it at 8000 rpm. After washing with 0.5 mL wash buffer AW1

and AW2 each, the nucleic acid adsorbed to the column is eluted with 50 μ L H_2O .

Amplification

5 A small fragment of parvo B19 DNA is amplified by addition of 20 μ L extract to 30 μ L Mastermix.

(Perkin Elmer, TaqMan PCR Core Reagent Kit with 5 μ L 10xTaqMan Buffer, 14 μ L 25mM $MgCl_2$, 4 μ L 2.5mM dNTPs, 1.5 μ L 10 μ M PT1.f(5'GACAGTTATCTGACCACCCCA3') (Seq. ID Nr. 1), 1.5 μ L 10 μ M PT1.r(5'GCTAACTTGCCCAGGCTTGT3') (Seq. ID Nr. 2), 1 μ L 5 μ M PT1.p(5'6-FAM-CCAGTAGCAGTCATGCAGAACCTAGAGGAGA-TAMRA3') (Seq. ID Nr. 3), 1 μ L Tween 20 (1%), 1.25 μ L gelatin (2%), 0.5 μ L AmpErase UNG (1U/ μ L), 0.25 μ L AmpliTaq Gold (5U/ μ L).

15 The amplification takes place under the following conditions in an ABI prism 7700:

1. AmpErase UNG reaction 2 min, 50°C
2. Initial denaturing 10 min, 95°C
3. Cycles of 15 sec at 95°C and 1 min at 58°C.

20 The PCR is evaluated by the increase of fluorescence during the PCR. The threshold value (i.e., the cycle at which the signal increases above the ground noise) serves for quantification. The threshold values of a standard series of a linearized plasmid with a cloned fragment of parvo B19 genome in this case serves as the baseline for measuring the parvo B19 concentration in the extract of the screening pool.

25 If the number of genome equivalents measured in the sample lies below the limit value of 10^4 GE/mL in the screening pool, the samples contained in this pool are cleared for use. On the other hand, if a value over the limit value is reached, the screening pool is resolved.

Resolution

The 24 subpools of a screening pool are diluted 1:200 in H₂O (distilled) in several steps by means of a robot and used without extraction in the TaqMan PCR.

5

Amplification of the resolution

A small fragment of parvo B19 DNA is amplified by addition of 20 μ L subpool dilution (corresponds to 0.1 μ L of the subpool) to 30 μ L Mastermix.

(Perkin Elmer, TaqMan Gold Kit with 5 μ L 10xTaqMan buffer, 14 μ L

10 25mM MgCl₂, 4 μ L 2.5mM dNTPs, 1.5 μ L 10 μ M
PT1.f(5'GACAGTTATCTGACCACCCCA3'), 1.5 μ L 10 μ M
PT1.r(5'GCTAACTTGCCCAGGCTTGT3'), 1 μ L 5 μ M PT1.p(5'6-FAM-
CCAGTAGCAGTCATGCAGAACCTAGAGGAGA-TAMRA3'), 1 μ L Tween
20 (1%), 1.25 μ L gelatin (2%), 0.5 μ L AmpErase UNG (1U/ μ L), 0.25 μ L
15 AmpliTaq Gold (5U/ μ L)).

The amplification takes place under the following conditions in an ABI prism 7700:

1. AmpErase UNG reaction 2 min, 50°C
2. Initial denaturing 10 min, 95°C
- 20 3. Cycles of 15 sec at 95°C and 1 min at 58°C.

Evaluation

The PCR is evaluated by the increase of fluorescence during PCR. By determining the threshold value (i.e., the cycle at which the signal rises above
25 the ground noise) in comparison with the values of a known standard series that was amplified at the same time, the number of genome equivalents in the extract of the screening sample is determined.

Since only samples with a content of at least 10⁴ GE/mL can produce a signal if the supply is 0.1 μ L material, all individual donations recognized as
30 positive in the resolution are discarded and the residual are released for use.

Claims

1. A method for producing a pool of quality assured biological samples, comprising:
 - taking aliquots from the biological samples;
 - 5 - combining the collected aliquots into a screening pool;
 - testing the screening pool for the presence of or the content of genome equivalents of microorganisms by a first nucleic acid amplification process which has a detection limit, DL-1, for the microorganisms to be tested;
 - dividing the screening pool into screening subpools by recombining the
10 collected aliquots into smaller pools, if a preset limit value of genome equivalents is exceeded in testing the screening pool,
 - retesting the screening subpool for the presence of or the content of genome equivalents of the microorganisms that are to be tested by means of a second nucleic acid amplification process, which has a selected detection limit
15 DL-2, for the microorganisms to be tested, where $DL-1 < DL-2$;
 - eliminating samples that exceed DL-2; and
 - combining remaining samples to produce said pool of quality assured biological samples.
2. The method of Claim 1, wherein said nucleic amplification method is
20 PCR.
3. The method of Claim 1, wherein said microorganism to be tested is a virus.
4. The method of Claim 1, wherein said microorganism to be tested is selected from the group consisting of a hepatitis virus, a retrovirus, and a
25 parvovirus.
5. The method of Claim 1, wherein said limit value in testing said screening pool is set between DL-1 and 10^5 genome equivalents/mL.
6. The method of Claim 1, wherein said biological samples are selected from the group consisting of individual blood donations, plasma, serum and cell
30 culture batches.

7. The method of Claim 1, wherein said screening pool is formed of 512 biological samples and is divided into 24 subpools.

8. The method of Claim 1, wherein said additional nucleic acid amplification method is carried out immediately after said first nucleic acid amplification method.

9. A quality assured pool of biological samples obtained by the method of Claim 1.

10. A pharmaceutical composition which comprises a pool of Claim 9; and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition as set forth in Claim 10, which comprises at least one component selected from the group consisting of plasma proteins, and recombinantly produced proteins.

12. The method according to Claim 4, wherein said hepatitis virus is selected from the group consisting of HAV, HBV, HCV, HDV, HEV and HGV.

13. The method according to Claim 4, wherein said retrovirus is selected from the groups consisting of HIV-1 and HIV-2.

14. The method of Claim 4, wherein said parvovirus is parvo B-19.

15. The method of Claim 5, wherein said limit value is set at about 10^4 genome equivalents/ml.

16. The composition according to Claim 11, wherein said plasma protein or recombinant protein is an enzyme.

SEQUENCE LISTING

<110> Baxter Aktiengesellschaft
Zerlauth, Gerold
Gessner, Matthias
Koettnitz, Karl
Gross, Patricia

<120> Patent concerning a production method for a pool of
biological sample

<130> 236PCT

<140> A1443/99
<141> 1999-08-20

<150> PCT/EP96/12345
<151> 1996-12-31

<160> 3

<170> PatentIn Ver. 2.1

<210> 1
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Beschreibung der künstlichen Sequenz:PCR primer

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 1
gacagttatc tgaccacccc ca

<210> 2
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Beschreibung der künstlichen Sequenz:PCR primer

<220>
<223> Description of Artificial Sequence:PCR primer

22

<400> 2

gctaacttgc ccaggcttgt

20

<210> 3

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Beschreibung der künstlichen Sequenz:PCR primer

<220>

<223> Description of Artificial Sequence:PCR primer

<400> 3

ccagtagcag tcatgcagaa cctagaggag a

31

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

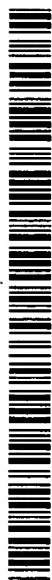


(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/14593 A3

- (51) International Patent Classification⁷: C12Q 1/70
- (21) International Application Number: PCT/EP00/07892
- (22) International Filing Date: 14 August 2000 (14.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
A 1443/99 20 August 1999 (20.08.1999) AT
- (71) Applicant: BAXTER AKTIENGESELLSCHAFT
[AT/AT]; Industriestrasse 67, A-1220 Vienna (AT).
- (72) Inventors: ZERLAUTH, Gerold; Sobieskigasse 42/9, A-1090 Vienna (AT). GESSNER, Mathias; Johann Nestroygasse 12/19, A-2301 Gross Enzersdorf (AT). KOETTINITZ, Karl; Bockfliesserstrasse 184/4, A-2232 Deutsch Wagram (AT). GROSS, Patricia; Heiligenstaeterstrasse 33/23, A-1190 Vienna (AT).
- (81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- (88) Date of publication of the international search report:
20 September 2001
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 01/14593 A3

(54) Title: A METHOD FOR PRODUCING QUALITY ASSURED BIOLOGICAL SAMPLE AND COMPOSITION CONTAINING THE SAME

(57) Abstract: A method for producing a pool of biological samples that is quality assured with respect to the load with microorganisms, using a nucleic acid amplification method, in which a screening pool is tested with a high sensitivity nucleic acid amplification method and can be divided into screening subpools, which are tested with a less sensitive nucleic acid amplification method, where individual samples can be picked out and eliminated, is described.

INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/EP 00/07892

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, EMBASE, BIOSIS, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>MCOMISH F ET AL: "DETECTION OF PARVOVIRUS B19 IN DONATED BLOOD: A MODEL SYSTEM FOR SCREENING BY POLYMERASE CHAIN REACTION" JOURNAL OF CLINICAL MICROBIOLOGY, US, WASHINGTON, DC, vol. 31, no. 2, 1 February 1993 (1993-02-01), pages 323-328, XP000577180 ISSN: 0095-1137 page 324, left-hand column; figure 1</p> <p style="text-align: center;">--- -/--</p>	1-8, 12-15

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

19 March 2001

Date of mailing of the international search report

30/03/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Gabriels, J

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/EP 00/07892

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROTH WILLI KURT ET AL: "Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting." LANCET (NORTH AMERICAN EDITION), vol. 353, no. 9150, 30 January 1998 (1998-01-30), pages 359-363, XP002163025 ISSN: 0099-5355 page 361 -page 362 ----	1-8, 12-15
Y	SCHOTTSTEDT V ET AL: "PCR for HVC, HCV and HIV-1 experiences and first results from a routine screening programme in a large blood transfusion service." BIOLOGICALS, vol. 26, no. 2, June 1998 (1998-06), pages 101-104, XP002163151 ISSN: 1045-1056 page 102 -page 104 ----	1-8, 12-15
Y	EP 0 922 771 A (CENTEON PHARMA GMBH) 16 June 1999 (1999-06-16) cited in the application claims 1-7 -----	1-8, 12-15

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 9-11,16

Present claims 9-11, and 16 relate to biological samples defined by reference to a desirable characteristic or property, namely a quality assured pool of biological samples.

The claim covers all biological samples having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a limited number of such biological samples. In the present case, the claims so lack support and, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the biological sample by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the method for screening the biological samples.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/07892

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0922771 A	16-06-1999	DE 19752898 A	05-08-1999
		JP 11225797 A	24-08-1999
<hr/>			